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13. ABSTRACT (Maximum 200 words)

The overall objective of this research program is to develop new approaches to predict the systemic toxicokinetics of chemicals utilizing in vitro experimental model systems and biologically-based kinetic (BBK) models. The focus of the proposed research is to test the hypothesis that in vitro measurements of membrane transport parameters in hepatic membrane vesicles are predictive of hepatic transport in vivo. Although this study is focused on transport processes in one particular organ, i.e. hepatic transport processes, these processes are a major controlling factor in determining systemic kinetics of many chemicals, particularly those which do not satify the "perfusion limited" condition usually assumed in many current PBPK models.

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FINAL REPORT - IN VITRO APPROACH TO PREDICTIVE TOXICOKINETICS

31 JAN 96

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OBJECTIVES:

The overall objective of this research program is to develop new approaches to predict the systemic toxicokinetics of chemicals utilizing *in vitro* experimental model systems and biologically-based kinetic (BBK) models. The focus of the proposed research is to test the hypothesis that *in vitro* measurements of membrane transport parameters in hepatic membrane vesicles are predictive of hepatic transport *in vivo*. Although this study is focused on transport processes in one particular organ, i.e. hepatic transport processes, these processes are a major controlling factor in determining systemic kinetics of many chemicals, particularly those which do not satisfy the "perfusion limited" condition usually assumed in many current PBPK models.

The first phase of the proposed three year research project is to develop a predictive paradigm for hepatic transport kinetics in the intact animal based on the rat hepatic membrane vesicle model. The specific goals are:

(1) To investigate the transport kinetics of a "set of reference chemicals" (cadmium, benzoic acid and trichloroacetic acid) in the rat hepatic sinusoidal membrane vesicle model and derive transport parameters for these chemicals,

(2) To utilize the isolated perfused rat liver model to determine scaling factors to convert transport parameters derived from the vesicle model (P = mass transport per unit concentration per unit area of membrane per unit time) to appropriate parameters for tissue kinetics in the BBK model (PA = mass transported per unit concentration per unit time),

(3) To incorporate the experimentally derived kinetic parameters into a BBK model for the rat and simulate *in vivo*, systemic kinetics, and

(4) To compare predicted kinetics with experimental, *in vivo*, rat kinetic data to refine the predictive paradigm.

Having established the predictive paradigm, the primary hypothesis will be tested by determining the membrane transport properties of a "second set of test chemicals" (phthalic acid, catechol and acetylsalicylic acid) in the hepatic membrane vesicle model and using the paradigm to predict *in vivo* kinetics. Both qualitative and quantitative criteria will be used to test goodness of fit.

This project is the first step in a series of planned studies to develop techniques to incorporate transport parameters derived from various *in vitro* models into BBK models. The long range goal is to utilize these techniques to predict *in vivo* toxicokinetics of chemicals in humans. The rat studies described here using *in vitro* models to predict *in vivo* toxicokinetics form the basis for the *in vitro* approach to predicting human kinetics.

STATUS OF EFFORT

Dr. Frazier, the Project Director for F49620-95-1-0104, was appointed to an Air Force ST position in November, 1995. He is stationed at OL AL HSC/OET, WPAFB. Consequently, his AFOSR project, administered through Wright State University, was officially transferred to AL/OET and combined with the Predictive Toxicology project (2312A202) already in place at AL/OET (previously administered by Dr. Jeff Fisher). Dr. Frazier is now the Project Director for the combined project. This report constitutes the final report for the original project. Subsequent progress reports will encompass the combined Predictive Toxicology project. The scope of both projects remain unchanged.

This progress report will discuss developments since the last annual progress report provided in December 1995 (see Appendix 1).

ACCOMPLISHMENTS/NEW FINDINGS

The main accomplish during this period is the completion of trichloroacetic acid (TCA) kinetic studies in the IPRL system. Rat livers were isolated from 200 g Fischer 344 rats and incubated in the computer controlled perfusion apparatus for a control period of 1 h. At the end of the control incubation, radiolabelled TCA was added to the perfusion medium (PM) to an initial concentration of 250 μM. This concentration was selected to correspond to the initial plasma concentration observed in vivo when rats were injected i.v. with 10 mg/kg TCA. The kinetic of TCA were observed for a period of 2 h by collecting perfusion medium samples at 2, 5, 10, 15, 30, 60, 90, 120 min and detecting TCA associated radioactivity by liquid scintilation counting. Bile was also collected over 30 min intervals throughout the incubation and assayed for TCA associated radioactivity. At the end of the 2 h incubation in the presence of TCA, the liver was removed, homogenized and assayed for TCA. Liver tissue samples were taken for histopathology (both light and electron microscopy). No evidence of TCA toxicity at the morphological level was observed.

The experimental data suggest that TCA rapidly reaches a dynamic equilibrium between the PM and the liver tissue. Throughout the remainder of the study the concentration in the incubation medium remained constant. This observation was not anticipated since TCA is a charged molecule at pH 7.4 and was expected to exhibit diffusion limited characteristics. The observed distribution between the PM and the liver suggested that protein binding may be playing a role in the dynamic equilibrium established (see below). TCA was eliminated in the bile. The rate of elimination in the bile decreased over the 2 h incubation with the highest amount eliminated during the first 0.5 h. The total amount eliminated in the bile over the 2 h incubation was less than 1 % of the administered dose. The concentration of TCA in the liver was determined at the end of the experiment and was lower than predicted assuming a totally passive distribution between PM and intracellular water spaces. Again this suggests protein binding in the PM may be important.

The results obtained to date suggested several additional studies. First, the issue of protein binding is being investigated. Preliminary data from protein binding studies using centrifigal

ultrafiltration techniques indicate a significant fraction of the TCA in PM is bound to protein. The only protein present in the PM is bovine serum albumin (BSA), which is presumably responsible for the effect. Using a Scatchard analysis, the binding capacity and binding affinity for the TCA-BSA complex has been estimated. Further studies investigating TCA binding in rat plasma and in liver tissues are scheduled. Additional studies designed to explain the decrease in biliary elimination of TCA over the course of the experiment are underway. Elimination of bromosulfophthalein (BSP), a well studied chemical that is excreted in the bile, will be investigated in the IPRL to determine whether the decrease in biliary elimination is an artifact of the experimental preparation. If this possibility is ruled out, we will investigate whether TCA itself modulates biliary excretion of BSP. This would determine whether TCA specifically inhibits transport of chemicals into bile. We already know that TCA has no effect on formation of bile. It is anticipated that these studies will take several weeks, but the results will allow us to better understand the IPRL preparation and improve our ability to interpret kinetic data derived from this *in vitro* model system

INTERACTIONS/TRANSITIONS

Poster Presentation SOT Annual Meeting, March, 1996 - Frazier, J.M. and Toxopeus, C. A biologically based kinetic model for the isolated perfused rat liver. (Appendix 2)